

# life lab

PRODUCTS, INFORMATION, AND SCIENTAINMENT  
ISSUE 23 | SPRING 2018

## Technology Propelling Breakthroughs

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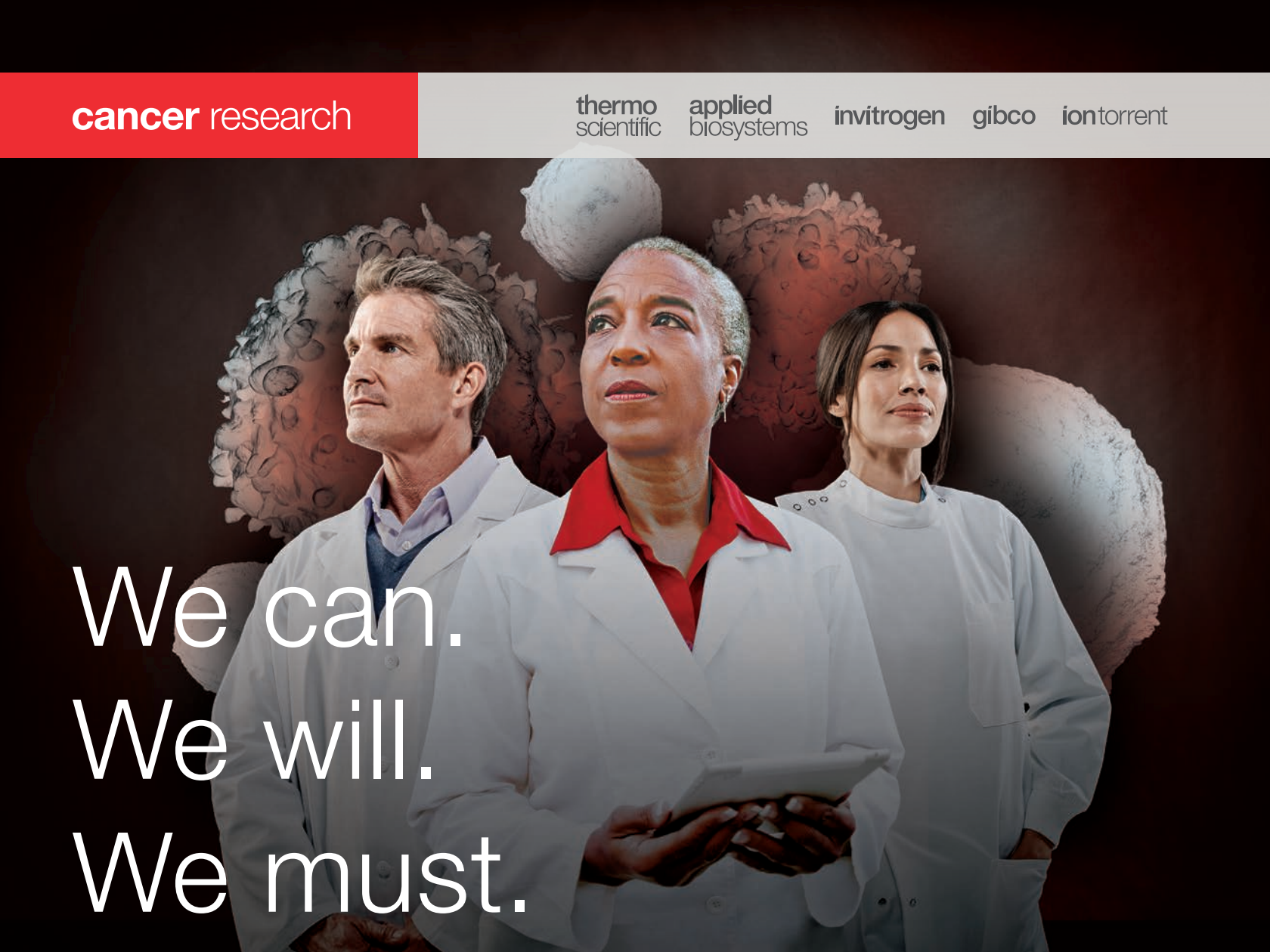
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S C I E N T I F I C



# IMPACTING THE NATURE OF CANCER RESEARCH

From machine learning predictions to personalized cell-based therapeutics—technologies propel cancer research



**Dipanjan Chowdhury, PhD,**  
Associate Professor of  
Radiation Oncology,  
Harvard Medical School  
[chowdhurylab.dana-farber.org](http://chowdhurylab.dana-farber.org)

Cancer research is being fundamentally changed in both experimental execution and data analysis.

The introduction of low-cost, high-throughput DNA sequencing has quickened the data-generating process. In a single day, sophisticated instruments run experiments transversing thousands of nucleic acids. As generating data is no longer a physically arduous process, the challenge now is finding the full meaning behind the data sets.

Dr. Chowdhury is creating a new blood test to rapidly detect early-stage ovarian cancer. This test detects small noncoding RNAs called microRNAs within the blood sample. Though microRNAs (defined as 22 base pairs) are smaller in size as compared to genes, there are still more than a thousand of these sequences located within the human cell. Working with microRNAs is no less complicated than a genome library—and data is rapidly generated with the same sequencing technology.

Chowdhury says, “Machine learning teaches the computer to recognize patterns. Instead of asking which individual genes contribute to ovarian cancer, we are looking for a signature combination of microRNAs.”

Chowdhury uses machine learning to leverage the sheer amount of data produced by sequencing blood samples from a patient with ovarian cancer. “We can feed the computer information from the thousands of microRNA molecules. We use statistics to understand the probability of microRNA levels found in patients with ovarian cancer versus patients without cancer cells or benign tumors. We don’t know how much of the combination of microRNAs contributes to the cancer itself. The algorithm gives the probability score of the risk of having ovarian cancer.”

Solving the data analysis bottleneck in cancer research may happen with machine learning, according to Chowdhury. He says, “I do believe the technology can be used in other cancers to produce similar success.”

In addition to machine learning, life science researchers are experimenting with ambitious therapeutic projects, especially in the development of personalized medicine.

Immunotherapeutics like chimeric antigen receptor (CAR) T cells genetically modify

the patient’s own cells to target and destroy cancer cells found in leukemia. Gene editing is the key technology to generate CAR T cells for use in immunotherapy.

CAR T cell therapy has shown promise in treating leukemia, but many factors remain that hinder its application to other types of cancer. Challenges include the lack of unique antigens on solid tumor cells and the difficulty that T cells have in infiltrating the solid tumor itself.

However, these problems may be addressed through improved antigen detection, changing gene editing strategies, and the use of more sophisticated technologies. Multiple academic and pharmaceutical laboratories are now working on improving the technology behind CAR T cell therapy.

Read about the successful use of CAR T cell therapy for a patient with leukemia on [page 16](#).

These technologies and therapeutics are in their infancy, but the potential of applying machine learning and personalized medicine to cancer treatment is promising.

Subscribe to the Life in the Lab blog to keep current on the latest breakthroughs in CAR T research at [thermofisher.com/lifeinthelabblog](http://thermofisher.com/lifeinthelabblog).

# BACK TO REALITY

## Extended reality takes a transformational role in the future of cancer research

might be scary reminders that technology can turn on us, they also shed light onto how powerful technology can be—integrating and improving our everyday lives.

Extended reality (XR) is a combination of the tangible, real world and new virtual enhancements, encompassing augmented reality (AR), virtual reality (VR), and mixed reality (MR)—and it's here to stay. From chasing Squirtle down the street to deciding where to fit your IKEA™ LACK coffee table, AR is not only a source of entertainment but a new-age convenience. Amazingly, VR is currently being used by GE Aviation to build jet engines, and medical students may soon be saving lives with procedures they learned from VR training. It may seem odd that a technology used for hunting pokéballs could be instrumental in solving some of the world's biggest questions, but there's an emerging use for all of the realities in how we research, treat, and monitor cancer progression.

Imagine a life without technology ... it's hard to even picture a time before dial-up. While futuristic shows like *Black Mirror*

XR and cancer research are intersecting at many points. At the recent Consumer Electronics Show™ (CES), the Best of CES 2018 Award went to the My Special Aflac Duck™ robot. This interactive toy helps kids through childhood cancer with its mixed-reality features to offer them much-needed comfort during treatment. At Weill Cornell Medicine, researchers are using VR and MR to investigate cancer-causing genetic mutations, hoping that it will enable doctors to select more effective, faster drug treatments. By performing 3D analysis of complex biological networks to study protein–gene relationships, scientists at Worcester Polytechnic Institute are leveraging mixed-reality technology like the HoloLens™ app to understand cancer proliferation and progression. Cancer Research UK is currently using VR for a 3D modeling project of tumors, to learn more about how cancer cells interact with one another within the tumor microenvironment.

These are just a few of the many scenarios where XR can be used in the fight against cancer and to advance cancer research. Charlie Brooker fan or not, the speed and impact these immersive experiences will have on cancer research in the next ten years is definitely something to keep your eyes—or goggles—on.

# TRANSFECTION TIPS FOR CANCER CELLS

What did the comedian and the cancer researcher have in common? They both needed help with their delivery. Here are three tips and tricks to help improve transfection of cancer cells.



Decreasing the serum content of the culture medium (to <10%) at the time of transfection is acceptable, but replace with complete growth medium within 4–24 hr posttransfection.



Antibiotics can be used during transfection.

Find even more tips and step-by-step transfection protocols for the most-studied cancer cell lines and cell types at [thermofisher.com/cancer/protocols](https://www.thermofisher.com/cancer/protocols)



Prior to flow cytometry, visualize cells under a brightfield microscope to verify dissociation following incubation with Gibco™ TrypLE™ reagent.

# CANCER BIOLOGY

## The importance of alternative splicing and noncoding RNAs

Gene expression profiling simultaneously compares the expression levels of multiple genes between two or more samples. This analysis can help scientists to identify the molecular basis of phenotypic differences and to select targets for in-depth studies, and it provides valuable insight into the role of differential gene expression in physiological and pathological processes.

Our comprehensive solutions for gene expression, from whole-transcriptome analysis to target confirmation, are supported by microarrays, sequencing, and real-time PCR technologies. Watch our recent on-demand webinars hosted by LabRoots to learn more.

## CANCER DEVELOPMENT AND BIOMARKER DISCOVERY



**Dr. Malte Buchholz,**  
Head of Basic Research, Clinic for Gastroenterology, University of Marburg, Germany

The interplay between microRNA (miRNA) and

messenger RNA (mRNA) has been found to be important in cancer development and progression. Simultaneous expression studies of miRNA and mRNA can be valuable in understanding molecular mechanisms that potentially have an underlying role in various diseases.

In this webinar, the speaker describes the technical verification of a novel method to reverse-transcribe and preamplify miRNA and mRNA from research samples containing a limited amount of serum.

[thermofisher.com/mirnowebinar](http://thermofisher.com/mirnowebinar)

## TRANSCRIPTOMICS TO DECIPHER CANCER DRUG RESISTANCE



**Yesim Gökmen-Polar,**  
PhD, Assistant Research Professor, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine

Computational biology,

combined with whole-transcriptome analysis, allows the characterization of differential gene expression signatures in response to treatment in resistant vs. nonresistant human-derived cancer cells. Many biomarkers, including alternatively spliced variants and long noncoding RNAs, have been associated with tumor pathogenesis and/or drug resistance. These represent candidates to help researchers to elucidate the biology of diseases, to identify the mechanisms for drug resistance, and potentially to design better-targeted therapies for precision medicine.

[thermofisher.com/drugresistance](http://thermofisher.com/drugresistance)

## Somatic variant detection to enable precision oncology research

Carcinogenesis is a complex process in which nucleic acid modifications play a significant role. DNA modifications include single mutations, deletions, insertions, duplications, and methylations. Somatic DNA mutations typically drive carcinogenesis by deactivation of proteins that normally inhibit tumorigenesis, or by constitutive activation of proteins that promote tumorigenesis.

Some somatic mutations like *KRAS/NRAS* occur in high frequency and can affect up to 40% of samples for certain cancer types. Since the sample is a combination of normal and tumor DNA,

accurate profiling of such somatic mutations requires a technology that can detect as little as 5% allele frequency. Sanger sequencing offers extended coverage for any variant along the entire amplicon and is particularly useful where limited genomic material is available.

The Applied Biosystems™ SeqStudio™ Genetic Analyzer, combined with high-quality consumables for Sanger sequencing, allows scientists to easily perform complex experiments to generate reliable and consistent data that are critical in clinical research applications.

## SANGER SEQUENCING MADE EASY— MOLECULAR DIAGNOSTICS WEBINAR

Sanger sequencing provides flexibility, accurate results, and a cost-effective solution for translational research. In this webinar, Dr. Luca Quagliata, senior director at the University Hospital of Basel, introduces the extended RAS research panel that uses Sanger sequencing technology. He specifically focuses on the advantages of the SeqStudio platform for capillary electrophoresis (CE) in routine molecular diagnostics, and looks to the future for potential applications of this platform in microsatellite instability, clonality evaluation, and detection of specific deletions.

[thermofisher.com/seqstudiooncologywebinar](http://thermofisher.com/seqstudiooncologywebinar)

## SUPERIOR CAPILLARY ELECTROPHORESIS PRODUCTS ALLOW YOU TO FOCUS ON THE SCIENCE



Applied Biosystems™ kits and reagents for Sanger sequencing are manufactured under robust ISO 13485 processes, so our customers receive high-quality products consistently. Additionally, our culture of continuous improvement and our 20+ years of experience in manufacturing help ensure that we provide reliable tools for scientists and researchers throughout the world.

[thermofisher.com/sangerworkflow](http://thermofisher.com/sangerworkflow)





# DIRECT-TO-CONSUMER **GENETIC TESTING**

How our sample prep solutions are helping to bring genetic testing direct to consumers

The direct-to-consumer (DTC) genetic testing market has grown over 30%, with millions of samples tested each year by just a handful of companies. This has led to an emerging need for cost-effective, fast, and automated sample prep solutions. In response, we have developed a versatile DNA isolation kit (the Applied Biosystems™ MagMAX™ DNA Multi-Sample Ultra 2.0 Kit) and a powerful bead processing module (the Thermo Scientific™ KingFisher™ Presto Purification System) for sample prep automation.

The KingFisher Presto instrument can be integrated with a small liquid handler such as a MICROLAB™ NIMBUS liquid handler to get a fully closed system for processing hundreds of samples a day. It can also be arrayed to work with robotic liquid-handling arms to process thousands of samples a day.

The MagMAX DNA Multi-Sample Ultra 2.0 Kit uses magnetic bead-based sample preparation technology for the isolation of genomic DNA (gDNA) from 50  $\mu$ L to 2 mL of whole blood, saliva, buffy coat, and buccal swabs. The resulting purified gDNA is ideal for a variety of downstream molecular biology applications such as qPCR, next-generation sequencing, and microarray analysis.

[thermofisher.com/kingfisher](http://thermofisher.com/kingfisher)





## LINEAR RECOVERY OF gDNA FROM SALIVA AND WHOLE BLOOD

The MagMAX DNA Multi-Sample Ultra 2.0 Kit processed on the KingFisher system is capable of efficiently recovering high-quality gDNA from as little as 50  $\mu$ L to as much as 2 mL of saliva or whole blood. This makes the MagMAX kit ideal for a large range of applications. Furthermore, the recovery of total gDNA using this kit is linear in relation to the input volume, meaning samples of different volumes can be compared directly without the use of sample normalization or reagent volume adjustments.

[thermofisher.com/magmaxultra](http://thermofisher.com/magmaxultra)



## VALIDATION FOR PHARMACOGENOMIC TESTING

One important downstream application for the high-quality gDNA isolated from the MagMAX DNA Multi-Sample Ultra 2.0 Kit is pharmacogenomics testing. For example, by sequencing the isolated gDNA, clinicians are able to determine an individual's clinical response to xenobiotics by looking at genetic variation in genes related to drug absorption, distribution, metabolism, and excretion (ADME). This prevents ineffectual or toxic drug treatments. When the MagMAX kit is used to isolate gDNA, >99% of relevant ADME genes are consistently identified through SNP screening.

[thermofisher.com/magmaxultra](http://thermofisher.com/magmaxultra)



# THE SKY'S THE LIMIT

Cancer research is challenging enough. Add in the scarcity of high-quality samples and target cells and the presence of unwanted inhibitors, and things get even harder. That's why we're inspired to develop technologies for you that bring superior sensitivity, specificity, and connectivity to help take your research to new heights.

## REVERSE TRANSCRIPTASES

### Invitrogen™ SuperScript™ IV One-Step RT-PCR System

- **Two-phase hot-start activation mechanism**—enables room-temperature setup and high specificity
- **Unmatched performance**—high sensitivity and target length, with the fastest protocol

[thermofisher.com/ssiv-onestep](http://thermofisher.com/ssiv-onestep)



### Invitrogen™ SuperScript™ IV VILO™ Master Mix

- **Improved C<sub>t</sub>**—up to 8 fewer cycles compared to other RT reagents
- **Superior linearity**—works with a broad range of input RNA for any RT-qPCR application

[thermofisher.com/4vilo](http://thermofisher.com/4vilo)



See the whole family of reverse transcriptases at [thermofisher.com/superscript](http://thermofisher.com/superscript)

## DNA POLYMERASES

### Invitrogen™ Platinum™ II Taq DNA Polymerase

- **Universal primer annealing**—reduces tedious optimization steps
- **Engineered Taq polymerase combined with leading hot start**—enables fast, robust, and highly specific PCR

[thermofisher.com/platinumiiataq](http://thermofisher.com/platinumiiataq)



### Invitrogen™ Platinum™ SuperFi™ DNA Polymerase

- **Exceptional fidelity**—100x more accurate than Taq DNA polymerase
- **Robust and versatile**—ideal for difficult targets

[thermofisher.com/superfi](http://thermofisher.com/superfi)



Cutting-edge and connected tools to make your research soar



## THERMAL CYCLERS

Applied Biosystems™ MiniAmp™ and MiniAmp™ Plus Thermal Cyclers

- Get superior reliability in an innovative, compact design with just the features you need
- Upgrade to the MiniAmp Plus Thermal Cycler for easy PCR optimization

[thermofisher.com/miniamp](http://thermofisher.com/miniamp)

### Cloud-enabled instruments

Boost productivity with secure remote access.

- Design and share protocols
- Schedule use of an instrument
- Start or stop a run
- Check run status

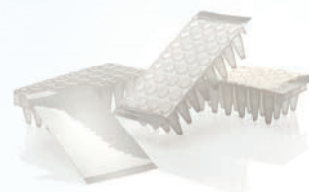
[thermofisher.com/connect](http://thermofisher.com/connect)

Thermo Fisher **Cloud** 

## PCR PLASTICS

Applied Biosystems™ plastic consumables have been designed to work seamlessly with Applied Biosystems™ instruments for optimal fit and performance.

[thermofisher.com/pcrplastics](http://thermofisher.com/pcrplastics)



## ELECTROPHORESIS SYSTEM

Invitrogen™ E-Gel™ Power Snap Electrophoresis System

- **Faster analysis**—sample loading to image capture in 15 minutes
- **Simple operation**—intuitive user interface with large touch screen and integrated operating system
- **Safer handling**—minimize handling of hazardous chemicals with Invitrogen™ E-Gel™ precast gel cassettes

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# CONNECTING YOUR **LABORATORY ESSENTIALS**

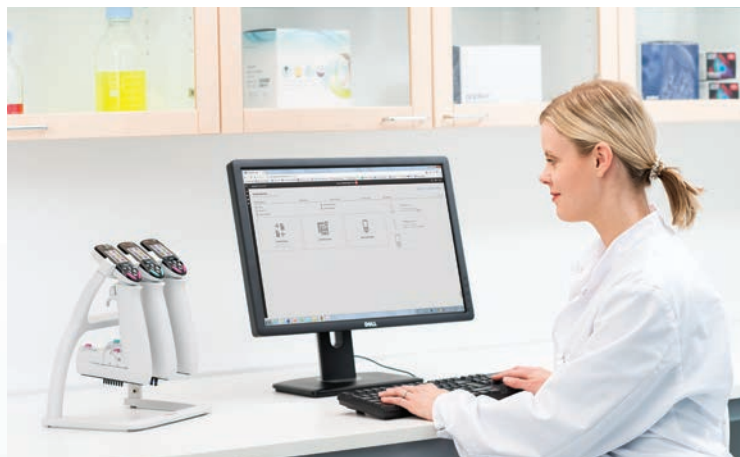
Thermo Fisher Connect continues to expand with new offerings

Researchers, benchtop scientists, and lab managers are seeking to maximize productivity amid increased regulatory requirements. We are addressing their needs with new connectivity offerings for essential lab equipment, including cloud-connected pipettes, freezers, biological safety cabinets, and incubators. Connecting via the secure, simplified Thermo Fisher™ Cloud will enable our customers to focus on their scientific work, rather than repetitive tasks, logistics, and tedious documentation management. In addition, a connected lab enables traditionally depreciating laboratory equipment to be transformed into appreciating assets.

## INTRODUCING MY PIPETTE CREATOR PIPETTING APP

With the My Pipette™ Creator app, manual pipetting customers can take advantage of efficient, centralized programming and sharing of protocols between pipettes and fellow colleagues. They can download and share preprogrammed protocols for many reagent kits from their computer. Using My Pipette Creator in conjunction with Thermo Scientific™ E1-ClipTip™ electronic pipettes offers a convenient and simplified way to help boost productivity, efficiency, and ultimately, research results.

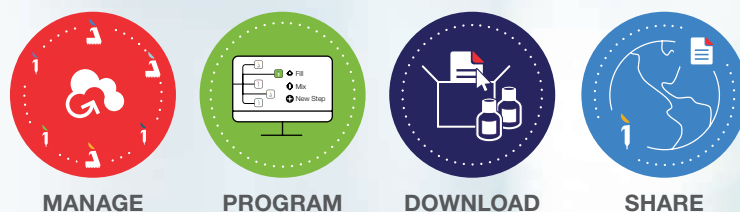
[thermofisher.com/mypipette](http://thermofisher.com/mypipette)



## COMING SOON: DEVICELINK HUB FOR COLD STORAGE BIOSAFETY CABINETS AND INCUBATORS\*

The Thermo Scientific™ DeviceLink™ Hub will enable customers using cold storage and incubation equipment to utilize the Thermo Fisher Cloud instead of manual, cumbersome data documentation processes. Available as a factory-installed option or a manual install for existing equipment, the DeviceLink Hub will assist with alarm notifications, temperature monitoring, managing status in real time, and historical data—all within the secure, simplified Thermo Fisher Cloud.

\* The content provided herein may relate to products that have not been officially released and is subject to change without notice.



Thermo Fisher **Cloud** 



invitrogen

# UNRAVEL

THE COMPLEXITIES OF CANCER

Scientific advancements happening now have the potential to advance our shared pursuit of life without cancer. We're committed to working with you, the research and medical communities, to accelerate cancer research by pioneering improvements in research that may impact tumor detection and selection, and response to therapy.

## PREDEFINED siRNA LIBRARIES FOR CANCER GENOME AND TUMOR SUPPRESSOR RESEARCH

Utilization of small interfering RNA (siRNA) remains the easiest and quickest way to perform gene knockdown studies. Highly effective Invitrogen™ *Silencer*™ and *Silencer*™ Select Human Cancer Genome and Human Tumor Suppressor siRNA libraries enable researchers to target and silence relevant genes and identify critical pathways in cancer research. These libraries provide high specificity and potency while yielding low off-target effects, empowering researchers to make breakthrough discoveries toward ending cancer in the future.

[thermofisher.com/sirnalibraries](http://thermofisher.com/sirnalibraries)



## LENTIARRAY CRISPR LIBRARIES ADVANCING CANCER RESEARCH

The Invitrogen™ LentiArray™ CRISPR Human Cancer Biology Library is a collection that targets some of the most common genes involved in the development of cancer. The gene targets within the LentiArray CRISPR Human Cancer Biology Library were selected using the most up-to-date genome databases, including the NCBI RefSeq database, and cross-referenced to the Gene Ontology (GO) Consortium database and/or the HUGO Gene Nomenclature Committee (HGNC) as well as The Cancer Genome Atlas (TCGA). The guide RNA (gRNA) designs for each target were then created using a proprietary design algorithm developed by the scientists at Thermo Fisher Scientific.

[thermofisher.com/crisprlibraries](http://thermofisher.com/crisprlibraries)

## TARGETING THE CAUSES OF INFANT BRAIN TUMORS

At Northwestern University, researchers are studying pediatric brain tumors and pediatric embryonal tumors with emphasis on atypical teratoid rhabdoid tumors (AT/RT), which are known for their extraordinary aggressiveness and resistance to therapy. In April 2017, a compelling paper was published in *Pediatric Blood & Cancer* that exemplifies use of LentiArray CRISPR libraries to identify polo-like kinase 4 (PLK4) as a novel and key therapeutic target for malignant rhabdoid tumors. (*Pediatr Blood Cancer*. 2017;64:e26551)

As a follow-up to their work, the team published an article in *Oncotarget* describing the quantification of a new therapeutic agent for PLK4. They leveraged Invitrogen™ SelectScreen™

kinase profiling services as well as SelectScreen™ drug safety and toxicity services (i.e., P450 and hERG screening services) to demonstrate the selectivity and safety profile of this promising compound. Other products from Thermo Fisher Scientific were also utilized, including Invitrogen™ PrestoBlue™ Cell Viability Reagent, Applied Biosystems™ TaqMan® gene expression assays, and various media. (*Oncotarget* 8:111190-111212 (2017))

Both of these publications provide excellent case studies of how you can leverage our products and services across your entire drug discovery workflow, from target identification and drug discovery to drug optimization and initial safety evaluation.



# CANCER PATIENT FINDS **HOPE & REMISSION** IN CAR T CELL THERAPY

When the U.S. Food and Drug Administration (FDA) announced its approval for use of a new immunotherapy in August 2017, it was a breakthrough for precision medicine. The immunotherapy, used to treat a form of pediatric leukemia, became the first FDA-approved, commercially available chimeric antigen receptor (CAR) T cell therapy.



This particular form of therapy uses Gibco™ Cell Therapy Systems (CTS™) Dynabeads™ technology, developed by Thermo Fisher Scientific, to activate and expand T cells that have been genetically engineered to recognize and fight cancers. It all starts with the patient's own cells, which are infused back into them after the process is complete.

Nicole Gularte's cells took this same journey, and today she remains cancer-free. But her road was far from easy. After being diagnosed with acute lymphoblastic leukemia (ALL) in 2010, and spending the next six years in and out of cancer treatments, the novel precision medicine approach was her last hope: she was exhausted, and she had exhausted all options.

Gularte, 32, answered questions about her journey for Life in the Lab.

**Life in the Lab:** At what stage in your battle with cancer did you first learn about CAR T cell therapy?

**Nicole Gularte:** After a relapse in 2014, my doctors at Stanford University told me that I would need a transplant and that I'd be looking at 3–5 more years of additional treatment with a lot of side effects and a low percentage of success. I kept pushing because there just had to be another option, and that's when they mentioned CAR T. But they told me that I wouldn't live to see CAR T treatments reach clinical trials and not to think about it because it would be a waste of my time. I left the hospital vowing to do my own research, and, in the meantime, I took part in another treatment that served as a bridge to CAR T.

**LITL:** Can you describe the experience you had with the interim treatment?

**NG:** We knew it would likely put me in remission quickly, but we also knew the cancer would come back. But it served an important purpose because it bought me time, and during that time, I pushed myself to pursue CAR T. Until that point, my traditional chemotherapy and radiation treatments were doing cumulative damage to my body



and I still suffer from side effects. They led to multiple knee surgeries, bone marrow biopsies, early menopause, and significant neurological damage—many side effects that still impact my daily life. There were no long-term side effects from the interim treatment, and the experience gave me confidence and hope for the CAR T therapy.

**LITL:** And you had to cross the country to be in position for a CAR T trial, correct?

**NG:** Exactly. When I was first in remission, I called the University of Pennsylvania, which had a CAR T trial on hold, but I had a strong feeling that it would eventually come back because of the initial studies and the success rates. So UPenn granted me an appointment and although they knew I would eventually relapse, I was healthy at the time. They collected and froze my cells for when either the FDA lifted the hold on the study or I relapsed, which is what I needed to do to qualify for the study.

**LITL:** And what was it like for you to play the waiting game before you finally qualified for the CAR T trial?

**NG:** For two years, from 2014–2016, I traveled, shared my story, and raised awareness while I was getting treatments. I had relapsed several times and the leukemia had spread into my eyes and spinal fluid, which kept me from being qualified for the study. And even though I was happy and still wanted to fight, the combination of the radiation

and the chemotherapy drugs that were injected in my spine every day was toxic, and it was diminishing my quality of life. That's when I decided to go on palliative care and stop treatment. When they gave me 3–5 weeks to live, I started planning my funeral. And since I was never baptized, my family and friends arranged for my baptism. I lived those weeks thinking they would be my last.

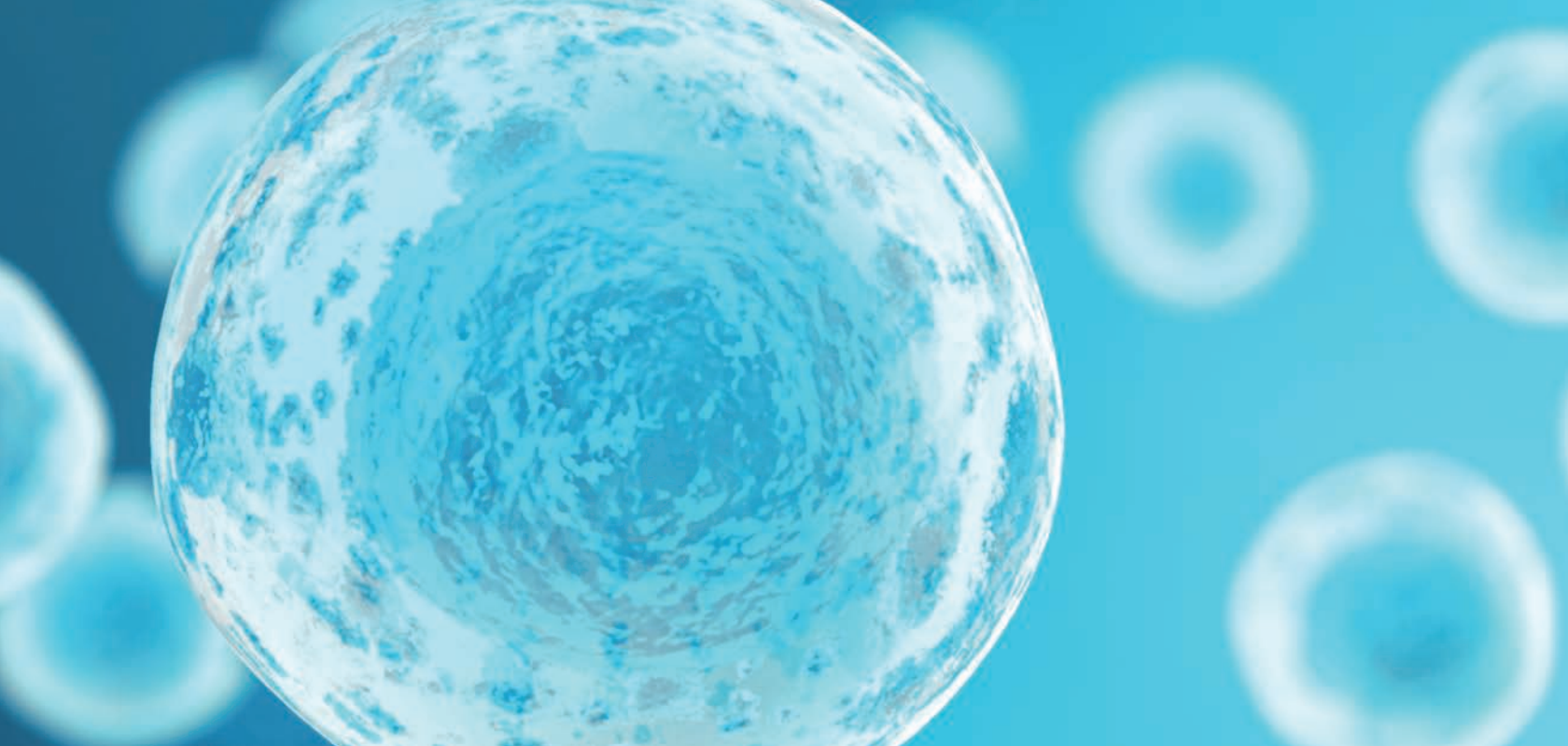
**LITL:** And then what happened?

**NG:** Miraculously, days before I was supposed to be dead, I had a final checkup and although the cancer didn't go away, my blood was good, and the leukemia was no longer detected in my spinal fluid, which qualified me for the study. The next week I flew out to UPenn and got the treatment. The experience was amazing and within 28 days, my leukemia was gone. It was crazy because I went from having it spread to my eyes, my spine, my lymph nodes, and my bone marrow to it being gone. I did experience some side effects—cytokine release syndrome (CRS) that led to an average fever of nearly 106 degrees over six days, and two small seizures—but they were short-term and relatively minor compared to the side effects from chemotherapy and radiation.

**LITL:** How are you feeling today? And what's next for you?

**NG:** Last October, I had my annual checkup, which confirmed that I have no leukemia cells anywhere. I've also become very involved with the Emily Whitehead Foundation and was honored to be a speaker at their inaugural gala, which included more than 30 CAR T survivors—all children, aside from me. After that event and my clean checkup, I've made my purpose in life to be a voice for children with cancer, and I'm now launching a West-Coast operation of the Emily Whitehead Foundation.

To learn more about Nicole Gularte's story, visit her blog: **The Inspired Hero: Leading The Way With Immunotherapy** at [nicolegularte.wordpress.com](http://nicolegularte.wordpress.com).



# CANCER AT THE CELLULAR LEVEL



## GIBCO CELL CULTURE HEROES— SPOTLIGHTING EVERYDAY WORK THAT SHINES

Heroes don't seek recognition, but they deserve it. Boldly determined and deeply committed, Gibco™ Cell Culture Heroes work tirelessly to lay the foundation for discoveries that may lead to cures. We want to show the world what they're doing.



## MEET OUR MARCH 2018 CELL CULTURE HERO

Jasmine Hughes, UC Berkeley, studying the mechanobiology of glioblastoma-initiating cells

Register for the next Cell Culture Heroes webinar and meet more of our heroes at [thermofisher.com/cellcultureheroes](http://thermofisher.com/cellcultureheroes)



## HALLMARKS OF CANCER

In 2000, cancer researchers Douglas Hanahan and Robert Weinberg described the six key changes that permit the transformation of a normal cell to a tumor cell; these features are called “the hallmarks of cancer.” In 2011, Hanahan and Weinberg updated that list by proposing four additional hallmarks. The acquisition of these hallmarks is crucial for the development of cancer cells. Learn about the ten hallmarks in our new Cancer Cell Culture Basics Handbook—visit the link below to obtain your free copy.

[thermofisher.com/cancer/cellculturebasics](https://thermofisher.com/cancer/cellculturebasics)

## VALIDATED TRANSFECTION PROTOCOLS FOR CANCER RESEARCH

Transfection is an essential step in many cancer research experiments. At the same time, cancer cells can be difficult to transfect. To save the time and frustration of optimization, we've developed step-by-step transfection protocols for the top researched cancer cell lines: breast, lung, liver, and prostate. These protocols cover everything from growing and passaging to transfecting cells. Now it's easier to get the results you're looking for the first time.

[thermofisher.com/transfectioncancer](https://thermofisher.com/transfectioncancer)



# THE IMAGE OF CANCER

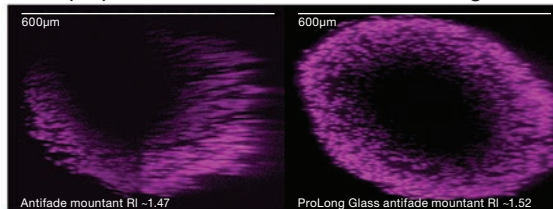
Deeper imaging of 3D cultures, organoids, and spheroids

Three-dimensional (3D) cultures, spheroids, and organoids are becoming increasingly popular for use as models in studying cancer biology. These 3D models improve scientists' ability to study the effects of treatments compared to traditional cell culture. Since these 3D cultures can be several hundred micrometers thick, imaging biological markers within cells that exist deep inside thicker samples becomes a challenge.

Dr. Douglas Richardson, director of imaging at a globally recognized biological imaging center, uses cortical neural organoids derived from induced pluripotent stem cells (iPSCs) as specimens for his imaging and microscopy research. These organoids have diameters of approximately 600  $\mu\text{m}$ : that is 75 times the width of your average 8  $\mu\text{m}$  cell. The image (above right) shows these cortical neural organoids identified by the Sir-Hoechst™ stain, imaged using a 25x LD-LCI oil-immersion objective on a Zeiss™ LSM 880 confocal microscope.

Talk about true 3D analysis—it is now possible to capture crisp images deep inside organoids from both lateral and axial directions. Invitrogen™ ProLong™ Glass Antifade Mountant is designed to allow scientists to capture crisp images of 3D cultures at a depth greater than any other commercial hard-set mountant.

**Axial (XZ) cross-sections of cortical neural organoids.**



ProLong Glass Antifade Mountant is a ready-to-use, hard-set mountant with a glass-matching refractive index of 1.52 after curing. This product minimizes refractive index mismatch between oil-immersion microscope lenses and 3D culture.

Advantages include:

- More than 3 times greater focal depth
- Up to 75% increased axial resolution
- Superior lateral resolution

It also delivers excellent signal-to-noise ratios and photobleaching protection across the spectrum for organic fluorophores and fluorescent proteins. This makes it ideal for producing sharp, bright, high-quality 3D images.

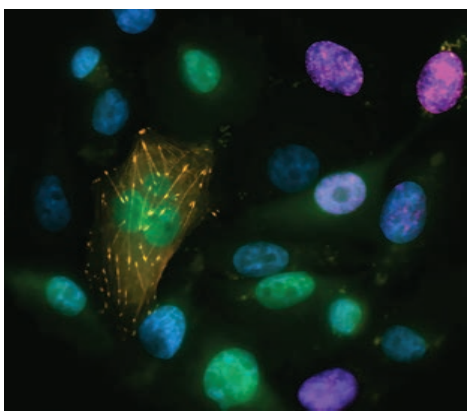
Compare and select the right hard-set antifade mountant for your next image at [thermofisher.com/prolong](http://thermofisher.com/prolong)



## Bringing light to cancer research

Cell proliferation assays provide a critical piece of the puzzle when evaluating cell health, genotoxicity, and the efficacy of anticancer drugs. Proliferation, however, is rarely assayed in isolation; other cell function probes are often used in concert with proliferation assays to provide a more informative picture of the state of the cell. To retain the detection of these cell function probes, Invitrogen™ Click-iT™ Plus EdU cell proliferation assays not only offer improved performance and an easier workflow, but are compatible with an even broader range of fluorescent probes—including GFP, RFP, and phycobiliproteins—allowing you to perform multiplex assays that provide a more informative picture of the state of the cell.

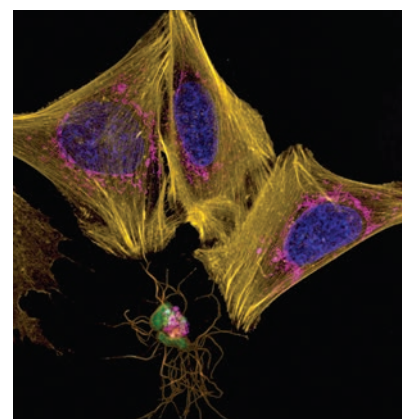
Discover more tools at  
[thermofisher.com/proliferation](http://thermofisher.com/proliferation)



Ironically, cell death mechanisms are critical for the survival of a multicellular organism. Apoptosis, or programmed cell death, is essential for proper growth and development by ridding the organism of unneeded cells and tissues, while also minimizing threats by destroying virus-infected or DNA-damaged cells. The inactivation of this programmed cell death is central to the development of cancer. The ability to study this key cancer process has become significantly easier with the Invitrogen™ CellEvent™ Caspase-3/7 Green Detection Reagent, a novel fluorogenic substrate for the detection of activated caspase-3 and -7. This reagent is compatible with high-content imaging and can be multiplexed with other probes for apoptosis and cell health, making it possible to study cell death in the context of other critical cellular functions.

Find additional resources at  
[thermofisher.com/apoptosis](http://thermofisher.com/apoptosis)

**Characterize proliferation more richly.** In this image of human malignant melanoma, not only can you differentiate proliferating cells (pink nuclei) from nonproliferating cells (blue nuclei), you can identify cells that are beginning to proliferate (blue nuclei with some pink) and cells that are actively dividing (blue-green nuclei with orange spindles).



**Multiplex imaging of apoptosis to get the whole picture.** U2OS human osteosarcoma cells experiencing etoposide-induced apoptosis, were stained first with CellEvent Caspase-3/7 Green Detection Reagent (green) to detect apoptosis, and then mitochondria were labeled with Invitrogen™ MitoTracker™ Deep Red FM dye (pink). Following fixation and permeabilization, actin was labeled with Invitrogen™ Alexa Fluor™ 546 Phalloidin (orange).

# PROLIFERATION ASSAYS

Uncontrolled cell proliferation is a hallmark of cancer. Researchers require reagents and instruments to measure dividing cells and quantitate newly synthesized DNA from challenging tumor samples.

## SPEEDING AHEAD WITH THE ATTUNE NXT FLOW CYTOMETER



The Attune NxT Flow Cytometer and the Attune NxT Autosampler with 384-well capacity.

Learn more about the configuration options of the Attune NxT Flow Cytometer at [thermofisher.com/attune](http://thermofisher.com/attune)

The Invitrogen™ Attune™ NxT Flow Cytometer is a benchtop analyzer that uses acoustic-assisted hydrodynamic focusing to precisely align cells prior to interrogation with one or more lasers.

The Attune NxT system provides cancer researchers with:

- No-wash, no-lyse options to minimize cell loss
- One to four lasers, with up to 16 detection channels
- Clog-resistant engineering

## STAINS FOR DNA SYNTHESIS

Measuring cell proliferation is a fundamental method for assessing cell health, determining genotoxicity, and evaluating anticancer drugs. Click-iT EdU assays accomplish this by providing:

- Quantitation of newly synthesized DNA
- Multiplexability with sensitive R-PE tandems and fluorescent proteins
- Fast detection—in as little as 60 minutes

Learn more at

<http://thermofisher.com/flow-cellproliferation>

## TRUSTED CELL CYCLE MARKERS

Cell cycle phases coordinate multiple proteins to synthesize DNA and divide cells. The Invitrogen™ portfolio of antibodies for flow cytometry provides:

- >10,000 antibodies for flow cytometry, including Invitrogen™ eBioscience™ conjugates
- A variety of fluorescent conjugates to build large multicolor panels
- Validated antibodies for quality results

Learn more at

[thermofisher.com/flowantibodies](http://thermofisher.com/flowantibodies)

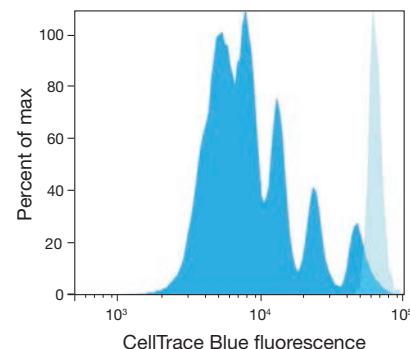
## DETECTION OF CELL POPULATIONS

Permanently label cells with fluorescent stains to trace generations or divisions without affecting morphology or physiology. Invitrogen™ CellTrace™ cell proliferation kits (right) offer:

- Labeling *in vitro* or *in vivo*
- Bright, single-peak staining
- Long-term signal stability

Learn more at

[thermofisher.com/flow-cellproliferation](http://thermofisher.com/flow-cellproliferation)



**CellTrace reagents are now available for detection of up to five colors including blue, violet, green (CFSE), yellow, and red.**

Cell labeling was performed for proliferation of harvested human T cells and followed for 5 days.

## AN INTERVIEW WITH JACOPO MORINI



**Jacopo Morini,**  
Postdoctoral Researcher,  
University of Pavia,  
UNIPV Department of Physics,  
Pavia, Italy

### Would you please tell our readers about yourself?

I work in the group of Radiobiology and Radiation Biophysics at the University of Pavia, directed by Professor Andrea Ottolenghi. Our group, formed of biologists and physicists, performs both experimental and theoretical research to elucidate the effects of ionizing radiation

on biological targets. If you are curious about our activities, please visit our website: [radiophys.unipv.eu](http://radiophys.unipv.eu).

### Can you describe the role of inflammation during cancer treatment?

Cancer treatment is routinely performed through different approaches: surgery, chemotherapy, and radiotherapy. Radiotherapy is different from surgery and chemotherapy as it releases high amounts of energy targeted to the tumor site, thereby minimizing the exposure to healthy tissues. However, during radiotherapy, irradiated tissues often show the cardinal signs of inflammation. In this scenario, ionizing radiation exposure may trigger the production of reactive oxygen species (ROS), the release of signal molecules (cytokines, chemokines), and the remodeling of the extracellular matrix, all leading to tissue modification (e.g. the onset of fibrosis). The inflammation may limit the amount of radiotherapy treatment given to the patient.

### Why did you choose to look at colorectal cancer as a model system?

During radiotherapy of colorectal cancer, as well as radiotherapy for other tumors (for example, prostate cancer), the healthy gut can be hit by radiation as a side effect.

Following radiation exposure, the function of the intestinal membrane can be compromised.

### What would be the role of flow cytometry in your work?

Radiation-induced damage includes that generated directly from X-rays on intracellular components, but also damage due to ROS production. One way we use flow cytometry is the analysis of DNA double-strand breaks (DSBs) by histone  $\gamma$ -H2AX staining. Depending on the type of radiation you use, there are differences in the way in which the energy is released into the cells, creating different patterns of DSBs. We merge the data produced by flow cytometry with images from immunocytochemistry to allow for a deeper investigation of DNA damage response dynamics. Another use is to correlate the onset of DNA damages with investigation of the cell cycle modifications after cell irradiation. The high speed of the Attune NxT [system] allows us to perform these formerly time-consuming experiments quickly, keeping data integrity. These are only a few examples, and there are no limitations to the role of flow cytometry in your work if you have enough creativity.

# DETECTION OF TUMOR MARKERS

Immunohistochemistry using Invitrogen antibodies:  
Superior performance through advanced verification

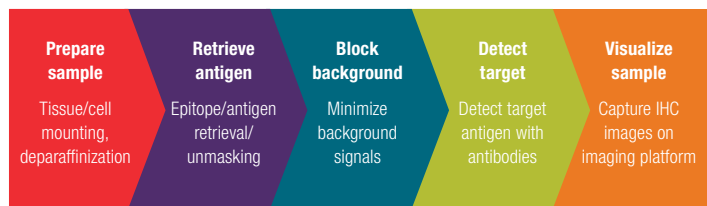
From sample prep through image acquisition, Thermo Fisher Scientific offers a broad portfolio of superior reagents, accessories, and instruments to help with every step of the immunohistochemistry (IHC) workflow.

One of the critical reagents for a successful IHC experiment is the detection antibody. We are striving to provide superior antibody performance by testing the specificity of Invitrogen™ antibodies in accordance with the newly adopted advanced verification testing methods for antibody validation.\* To help ensure superior antibody results, we've expanded our specificity testing methodology using a two-part approach for advanced verification.

**Part 1 – Target specificity verification:** verifies the antibody will bind to the correct target. Our antibodies are being tested using at least one of our specificity tests to ensure proper functionality in researchers' experiments.

**Part 2 – Functional application validation:** verifies the antibody works in a particular application(s) of interest.

To find out more about the two-part testing approach, go to [thermofisher.com/antibodyvalidation](https://thermofisher.com/antibodyvalidation)



Overview of the IHC process.



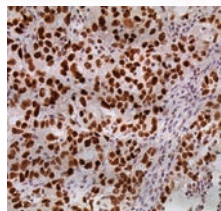
## TUMOR DETECTION

Cancer protein biomarkers are useful in applications such as IHC to identify where and to what degree cancer has affected healthy tissues. These proteins can be expressed in some cell or tissue types but not in healthy tissues. Antibody target verification can be determined by analyzing the relative expression of these proteins in different tissue types using IHC. For example, the tumor suppressor p53 protein is known to be strongly expressed in the nuclei of tumor cells within ovarian cancer tissue, compared to low expression seen in normal, healthy ovary tissue. Additionally, p53 expression is not detectable in other tissues, such as normal, healthy human liver. The IHC analysis of p53 expression in positive ovarian cancer and negative ovary and liver tissues shown at right demonstrates the specificity of the p53 antibody used for the detection. As expected, p53 is highly expressed (stained brown) in the nuclei of ovarian cancer cells, but expressed at a low level in normal ovary tissue and not expressed in normal liver tissue.

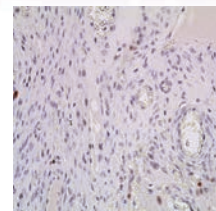
The same antibody verification and IHC workflow described can also be performed using immunofluorescence (IF). For fluorescence detection, the primary or secondary antibody is conjugated to a fluorophore that is detected by fluorescent microscopy. Recently introduced, Invitrogen™ Alexa Fluor™ Plus secondary antibodies offer high signal-to-noise ratios and superior brightness. Images to the right depict an example of IHC detection by IF using a p53 monoclonal primary antibody and an Alexa Fluor Plus 488 conjugated secondary antibody to visualize the relative expression of p53.

Learn more about these products at [thermofisher.com/IHC5steps](https://thermofisher.com/IHC5steps)

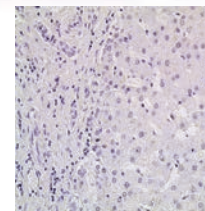
Ovarian cancer



Ovary

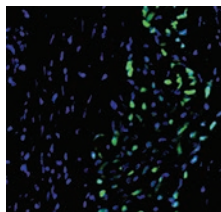


Liver

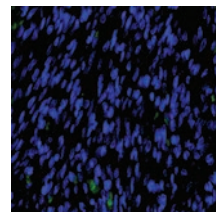


**Detection of relative p53 expression by IHC.** The specificity of anti-p53 monoclonal antibody (Cat. No. MA5-12571) was demonstrated by detecting relative p53 expression in ovarian cancer tissue (positive, left panel), normal ovary tissue (low expression, middle panel), and normal human liver tissue (negative, right panel). Detection was performed using a goat anti-mouse IgG (H+L) secondary antibody and Alexa Fluor Plus 488 conjugate (Cat. No. A32723). Tissues were imaged on the Invitrogen™ EVOS™ FL Auto Imaging Station.

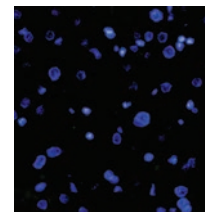
Ovarian cancer



Ovary



Liver



**IHC detection of p53 across tissues by immunofluorescence.**

The specificity of anti-p53 monoclonal antibody (Cat. No. AHO0152) was demonstrated by detecting relative p53 expression in ovarian cancer tissue (positive, left panel), normal ovary tissue (low expression, middle panel), and normal human liver tissue (negative, right panel). Images were taken on an EVOS FL Auto Imaging Station.

\* The use or any variation of the word "validation" refers only to research-use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. It does not ensure that the product(s) was validated for clinical or diagnostic uses.

# ACCELERATE YOUR RESEARCH ON GTPASES

For several decades, scientists worldwide have been studying how small GTPases play a role in cancer. Exciting research has demonstrated that small GTPases contribute to multiple cellular processes that could affect cancer progression, including cytoskeletal dynamics, cell cycle progression, transcriptional regulation, cell survival, and vesicle trafficking.

We are facilitating the study of active and assembled GTPase complexes with Thermo Scientific™ GTPase pull-down and detection kits.

These kits enable GTPase activation studies by preferentially enriching their active form. They contain a GST–protein-binding domain (PBD) fusion that is selective for active Rho, Ras, Rac1, Cdc42, Rap1, or Arf1. The protein-binding domains of the respective downstream effectors are expressed as GST fusion proteins. While immobilized on glutathione agarose resin, the protein-binding domain will bind active, GTP-bound GTPase from a cell lysate.

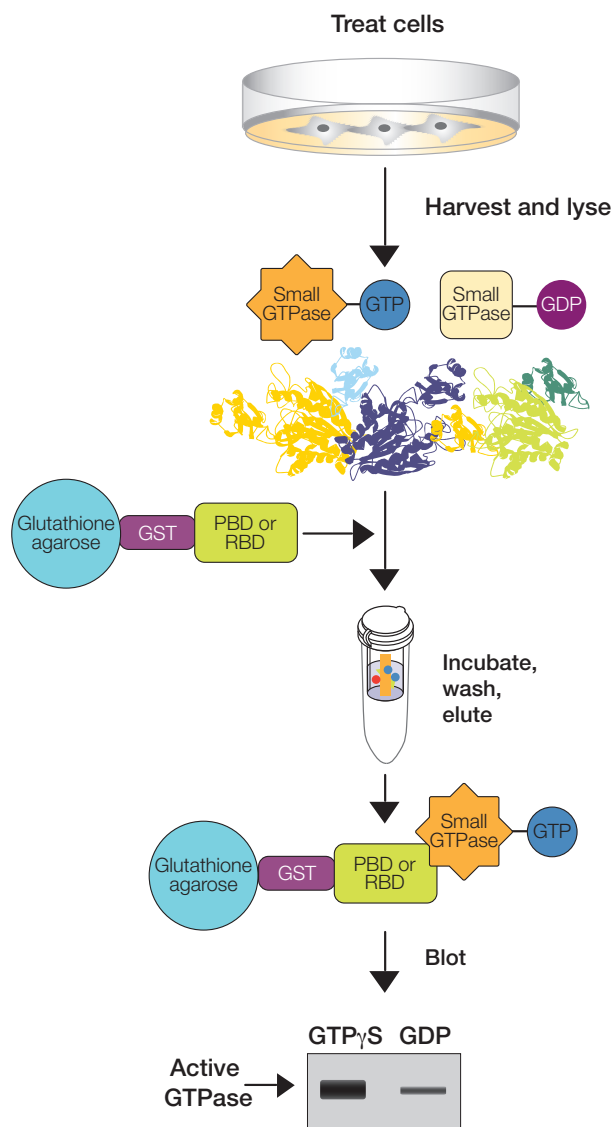
#### Applications:

- Follow activation of small GTPases during cell differentiation, migration, division, and cytoskeletal rearrangement
- Study the activation of GTPase during stress fiber formation
- Monitor activity after stimulation with growth factors
- Screen small-molecule inhibitors for their effects on activity

Our active GTPase pull-down and detection kits provide an effective way to monitor sensitive changes in GTPase enzyme function using time-dependent activity assays—helping scientists assess the involvement of small GTPases in several disease states such as cancer and metabolic disorders.

Learn more at

[thermofisher.com/gtpase-pulldown](http://thermofisher.com/gtpase-pulldown)



## EVERYDAY HERO

Roman Fischer, PhD,  
University of Oxford,  
Oxford, UK,  
Thermo Scientific TMT  
Research Award,  
Gold Level Recipient 2017

**Area of Research:** Dr. Fischer leads the Advanced Proteomics Facility of the Target Discovery Institute at the University of Oxford. He specializes in collaborative work ranging from basic research projects to discovery of new drug targets and markers in the clinic or even paleo-proteomic work. Currently Dr. Fischer and his team focus on the miniaturization of sample preparation methods to increase sensitivity and efficiency to access the deep proteome from limited clinical material.

**The challenge:** The deep proteome is now easily accessible in cultured cells. However, the detection of >2,000 proteins from limited biological material such as tissue sections or individually isolated cells of one phenotype (i.e., by sorting or laser capture microdissection) is still a major challenge, while presenting a good approximation to “single cell proteomics.”

**How he tackled it:** Dr. Fischer and his team used laser capture microdissection of individual Purkinje neurons to evaluate different sample preparation methods for their efficiency to identify a maximum of proteins from a minimal number of cells. Protein immobilization on microbeads and subsequent digestion (SP3 method) resulted in the highest number of identified proteins compared to standard in-solution digest, FASP, and iST methods.

**The happy result:** The SP3 method allowed the robust detection of >2,500 proteins from only 200 individual Purkinje cells. When the team combines this method with tandem mass tag (TMT) reagents and pre-fractionation, they anticipate the detection and quantitation of the deep proteome in different brain cell types within a specimen, allowing spatial proteome analysis with cellular resolution.

Learn more about the TMT Research Award program at [thermofisher.com/tmtgrant](http://thermofisher.com/tmtgrant)

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“In my institute, many customers come to me for speed and flexibility for different gene panels, tailored to disease areas of interest. **This is one of the most elegant workflows on the market today**, and I can now offer a range of applications and deliver the data in the fastest turnaround time in the market, at costs that are competitive.”

– Morten Dunø, Lab Manager; Molecular Genetic Laboratory, Department of Clinical Genetics; Rigshospitalet, Copenhagen University Hospital, Denmark  
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## NEW INNOVATION IN LIBRARY PREP

Ion AmpliSeq™ HD technology,\* a new development in library preparation, is coming later this year. This advanced technology will be ideal for ultralow-frequency variant detection from both liquid biopsy and FFPE samples, and will enable custom liquid biopsy panels.



\*The content provided herein may relate to products that have not been officially released and is subject to change without notice.



## TARGET MULTIPLE CANCER TYPES FROM A SINGLE VIAL OF BLOOD

The Ion Torrent™ OncoPrint™ Pan-Cancer Cell-Free Assay enables reproducible detection and analysis of tumor DNA and RNA across all major classes of somatic mutations (SNVs, indels, CNVs, and fusions) from a single vial of blood and from as little as 1 ng of nucleic acid input within two days.

Targeting over 50 genes across multiple cancer types (including lung, colorectal, breast, pancreatic, thyroid, and others), the cell-free total nucleic acid (cfTNA) assay is the latest addition to our rapidly growing portfolio of liquid biopsy NGS assays.

[thermofisher.com/cfna-assays](http://thermofisher.com/cfna-assays)



# BLAST FROM THE PAST

While we are highlighting technological advancements and sharing how they impact your research, we thought it might be fun to hop in a time machine and share some retro products that make life in your lab more entertaining.



Viewing an instructional video needn't be boring again with this fun **TV stand** for your tablet.\*

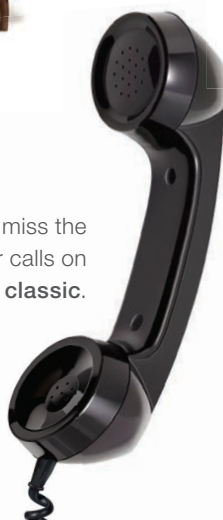


Share your vacation photographs with your colleagues on this cool **smartphone magnifier**.\*



Sharing your cell images in a presentation is simple but dry; make it more fun at events with a **custom reel viewer**.\*

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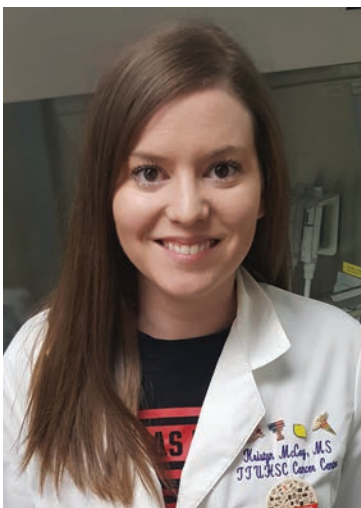


Follow us on social media and share photos of your retro lab equipment still in use today with **#lifeinthelab**

\* Product and photo courtesy of **uncommongoods.com**.

# BEHIND THE LAB COAT:

## MEET CANCER RESEARCHER KRISTYN MCCOY



**Kristyn McCoy,**  
**Medical Researcher at**  
**Texas Tech University Health**  
**Sciences Center**

**Everyone has a different story as to why they chose a career in science. What motivated you to become a scientist?**

I wanted to go into cancer research after being misdiagnosed with leukemia when I was 16. I had many appointments in hematology/oncology at Cook Children's Hospital in Fort Worth, Texas, where I was around many children much younger than me with cancer. Being in that environment, I knew I wanted to work in pediatric oncology; I just didn't know it would lead me to become a scientist. When in college, I worked as an undergraduate researcher and really enjoyed developing experiments. I liked the freedom

of research and working with the unknown. I love that every day is different. While the main focus of the lab is to create cancer models, the necessary steps are always changing. We are constantly looking for ways to improve our methods. I work with an amazing team that makes my job not feel like work.

**What is a typical day like in your lab?**

The lab where I work is home to the Children's Oncology Group Cell Line, the Xenograft Repository, and the Texas Cancer Cell Repository. We receive specimens from patients at diagnosis or relapse, from all types of cancers. We process these samples to try to extract the cancer cells, and then we grow them either *in vitro* (on plasticware) or *in vivo* (in an animal model called a xenograft; our lab uses mice). Our goal is to create as many cancer models as possible, and then characterize and maintain the cell lines and xenografts. We will use these models to test new chemotherapies or novel drug combinations. We openly distribute many of our cancer models for free. We want to build new collaborations with laboratories all over the world to help find a cure. Cancer is so complex and we need to work together on characterizing it, determining mechanisms of resistance, and finding the best option for treatment.

**You obviously enjoyed science from a young age, but you must have met challenges along the way. Do you have any advice for junior scientists facing challenges?**

I found it best to work through tough times with a good support system. I had roommates that were in my field of study. My friends that I would hang out with outside of class were also my colleagues. We had each other to help when studying or writing papers. A good support system could be your family, members of your church, individuals from clubs you're involved in, or getting involved in community service. Getting a bad grade also should not discourage you. I did not have a perfect GPA as an undergraduate and was still accepted into a graduate program. I was able to persevere with the support of my friends and family.

**Do you have a memorable breakthrough moment or favorite memory in the lab?**

A few years ago, we were working with UT Southwestern to create an assay to determine the human content of a xenograft tumor. The idea was created by UT Southwestern, but a small group of us at Texas Tech actually designed and optimized the assay. This test was important to develop to verify that the tumors grown in the mice are high-percent human, since mice can get cancer too.

**Why is your research important? What are the possible real-world applications?**

The work and research from the lab I work in is translational science. We develop and test a variety of chemotherapies for potential use in the clinic. We currently have three clinical-phase trials open to patients. Clinical trials are used to determine optimal dosing, safety, and efficacy of the drug in small groups of patients before the chemotherapy is approved to be used widely in the clinic or hospital. We get to hear about patients, mainly children, who have been on the chemotherapy developed in this lab who are cured, living a normal life, and going to college years after treatment.

**#IAmAScientist**

We want to hear what inspires you

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